gated ion channels (pLGICs) in the brain. The molecular details of this interaction including location of binding sites, conformational changes induced and residues involved in allosteric transduction remain largely unknown. We are using GLIC, a prokaryotic pLGIC to elucidate mechanisms underlying the action of the commonly used intravenous GA, propofol.
We individually introduced cysteines at seven sites in the TMD that frame the intra- (I201C in M1, V241C in M2 and T254C in M3), and inter-subunit (N238C, L240C and E242C in M2) cavities as well as the channel lumen (T243C in M2) in GLIC. Propofol slowed the rate of modification of L240C (inter-subunit) and increased the rate of modification of T254C (intra-subunit) suggesting that the extracellular end of the TMD undergoes propofol-induced structural motions that rearrange these cavities and change the local environment at these sites. An increase in modification rate of T254C makes it unlikely that it faces into the propofol binding site as suggested by a recent crystal structure of GLIC with bound propofol (Nury et al., 2011). Moreover, we found that perturbation of residues in the inter-subunit cavity and not those in the intra-subunit cavity caused propofol to potentiate GLIC currents rather than inhibit. Taken together our results show a significant role of the inter-subunit cavity in propofol modulation of pLGICs and reveal conformational changes associated with propofol's actions.

1066-Plat
X-Ray Structures of an Interfacial Potentiating Site for Alcohols and Anesthetics in a Pentameric Ligand-Gated Ion Channel
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Alcohol consumption produces a variety of undesirable behavioural and physiological effects in animals and humans and can eventually lead to addiction. Converging evidences suggest that its molecular mechanisms of action involve specific protein targets. Among these, pentameric ligand-gated ion channels (pLGICs) and especially GABA-A Receptor have been shown to be one of the main targets of ethanol in the central nervous system. Here we report the first atomic-resolution structure at 2.8 Å of ethanol bound to a member of the pLGIC family, the pH-gated prokaryotic homolog GLIC variant F14'A. This GLIC variant is potentiated by concentrations of ethanol similar to the ones effective in the vertebrate Glycine and GABA-A receptors (R. Howard et al., 2012). Comparison the ethanol-bound and apo structures of GLIC F14'A gives a rational and simple explanation to the potentiating effect of ethanol on these receptors by stabilizing the open form. Multiple-sequence alignments and homology structure models suggest that the ethanol binding-pocket identified in GLIC is also present in human Glycine and GABA-A receptors.

1067-Plat
ELIC Channel Conformational Changes Detected by 19F NMR
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Conformations of the pentameric ligand-gated ion channel (pLGIC) from *Erwinia chysanthemi* (ELIC)change at different functional states. Agonist binding to ELIC elicits channel opening for seconds or minutes before the channel goes to a prolonged desensitization state, in which the channel is closed in the presence of agonists. Our functional studies suggest that the general anesthetic propofol modulates ELIC negatively and inhibits ELIC channel current. Details of ELIC conformational changes upon agonist or anesthetic binding, however, have not been elucidated. In this study, we mutated two pore-ling residues (F247C and S229C) and a residue in the TM2-3 loop (L256C), labeled them with 2,2,2-trifluoroethanethiol (TET), and performed a series of ^{19}F NMR experiments on the samples. Several results are noteworthy. First, residues L256C and S229C have distinctly different ^{19}F NMR resonance peaks: the former appears as an overlap of two broad peaks, but the latter has four peaks with varied line widths, suggesting co-existence of different conformations. Second, upon adding the agonist propylamine to the samples, the ^{19}F NMR spectra of L256C show no significant changes, but the ^{19}F NMR spectra of S229C or F247C/S229C change profoundly, including the occurrence of a fifth peak. The fifth peak amplitude increases along with the increase of agonist concentrations (3, 10 to 18 mM) and a gradual decrease of the broad peak components. The data suggest a conformational shift in the pore and an increase in desensitization conformation due to long exposure of agonists. Third, the anesthetic propofol further increases the population of desensitization conformation in the S229C or F247C/S229C ELIC by ~4%. Together, we have demonstrated a powerful approach for investigating conformational changes of pGLICs under different stimulations or modulations. Supported by NIH (R01GM066358, R01GM056257, R37GM049202, and T32GM075770).

1068-Plat
Mechanism, Energetics, and Pathways for Association and Binding of Anesthetics to Membranes and Membrane Channels
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General anesthetics have, arguably, been one of the most important advances in medicine. Yet despite over a century of research, the mechanism of general anesthesia is still poorly understood. While it is well accepted that the biological targets of anesthetics are the ligand-gated ion channels of the Cys-loop superfamily, how anesthetics affect channel gating dynamics is unknown. Recently, bacterial homologues of these channels have been crystallized with anesthetics bound, making the problem more tractable to molecular dynamics. However, the lack of a widely available and reliable set of parameters for anesthetics has impeded progress. Utilizing a standard scheme, we parameterized four common modern anesthetics: desflurane, isoflurane, sevoflurane, and propofol. The quality of the generated parameters has been assessed against experimental bulk and solvation properties. In order to better understand how these anesthetics interact with membranes, we have also calculated the free energy profile for inserting each anesthetic into a POPC membrane utilizing the umbrella sampling method. All anesthetics show a distinct preference for the headgroup region of the membrane, with negligible free energy differences between bulk solution and the membrane core. Using the parameters developed, we simulated the interaction of desflurane and propofol with the bacterial ligand-gated ion channel, GLIC. In both cases, the anesthetic spontaneously releases from its binding site and exits the protein via the membrane. A triad of hydrophobic residues forms a gate securing the anesthetic in place and allowing the drug to exert its effects. Once these residues separate, the anesthetic quickly unbinds and exits the protein. Thus, we have observed a putative protein-binding pathway, which dictates that the anesthetic must first partition into the membrane before entering the protein in order to access the binding site and exert its effects.

Platform: Cardiac, Smooth & Skeletal Muscle Electrophysiology

1069-Plat
Repolarization Reserve Revisited: How the Transient Outward K Current can Promote Early after depolarizations (EADs)
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Introduction: EADs, an important cause of arrhythmias such as Torsades de pointes, are generally suppressed by outward currents. Here we use the dynamic clamp technique to show that the transient outward K current (I_{to}), a purely outward current, can paradoxically promote EADs.
Methods: Isolated patch-clamped rabbit ventricular myocytes were exposed to H_2O_2 (1 mmol/L) or hypokalemia (2.7 mmol/L) to induce bradycardia-dependent EADs. The dynamic clamp technique was used to inject a virtual I_{to} with programmable properties into the myocyte.
Results: H_2O_2 (n=17) or hypokalemia (n=11) induced EADs at a pacing cycle length (PCL) of 6 s, which were suppressed at PCL 1 s. Voltage clamp experiments revealed that substantial I_{to} was present at PCL 6 s, but was absent at PCL 1 s due to slow recovery from inactivation. Reintroducing a virtual I_{to} by dynamic clamp at PCL 1 s caused EADs to reappear when I_{to} parameters had moderate conductance (0.02-0.05 nS/pF), modest pedestal ($\leq$0.5 of peak I_{to}), and delayed fast inactivation (50-65 ms). In complementary experiments at PCL 6 s, the I_{to} antagonist 4-aminopyridine (2 mmol/L) suppressed EADs (n = 16), which were restored by reintroducing a virtual I_{to} by dynamic clamp. These findings were reproduced in computer simulations.
Conclusions: I_{to}, despite being a purely outward current, can promote EADs when its amplitude, kinetics and pedestal are in a critical parameter range. Simulations revealed that I_{to} lowers the AP plateau voltage into a range which slows activation of other time-dependent K currents while facilitating L-type Ca current reactivation. Thus, enhanced early repolarization paradoxically decreases late repolarization reserve, allowing EADs to emerge. These findings caution against I_{to} activation as an anti-arrhythmic strategy in settings of reduced repolarization reserve such as heart failure and long QT syndromes.

1070-Plat
The Contribution of CLC-1 and Kir Channels to the Resting Electrical Properties of Skeletal Muscle Fibers from Normal and Myotonic Mice
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The membrane resistance (R_m) of mammalian skeletal muscle fibers at rest has been typically assumed to be dominated by the chloride conductance (gCl). We